UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

MAY 9 1996

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Triclopyr

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and

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and

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THROUGH: Stephanie R. Irene Ph.D. Stephanie R. Irene
Acting Director, Health Effects Division (7509C) 5/8/96

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on August 09, 1995 to discuss and evaluate the weight-of-the-evidence on Triclopyr with particular reference to its carcinogenic potential. The CPRC concluded that Triclopyr should be classified as Group D - not classifiable as to human carcinogenicity. This decision was based on increases in mammary tumors in both the female rat and mouse, and adrenal pheochromocytomas in the male rat, which the majority of the CPRC believed to be only marginal.

(1)

SUMMARY

Administration of Triclopyr in the diet to ICR mice resulted in a statistically significant positive dose-related trend for mammary adenocarcinomas in female mice. Although increases occurred at all doses, statistical significance was not achieved by pairwise comparison to controls at any dose; however, at the highest dose (1250 ppm) the increase was borderline significant (p=0.051). The incidence of mammary adenocarcinomas in female mice at the highest dose (8%) exceeded the upper range of the historical data (4.7-5%). There were no tumors in concurrent controls. The majority of the CPRC considered the tumor response in the female mice to be marginal.

There were no apparent increased incidences of tumors reported for male mice. The dosing in the mouse study was considered to be adequate in both sexes.

Administration of Triclopyr in the diet to F344 rats resulted in increases in mammary adenomas, adenocarcinomas and combined adenoma/carcinoma in female rats at the highest dose only (36 mg/kg/day). The combined adenoma/carcinoma at the highest dose was statistically significantly increased; there were also statistically significant positive dose-related trends for carcinomas, alone and combined. However, the CPRC questioned the significance of the trends, since only 16 at the low dose and 19 animals at the mid dose were microscopically examined for mammary tumors vs 49 in the control group and 50 at the high dose. The incidence of mammary adenocarcinomas at the high dose (8%) exceeded the upper range for historical controls (0-4%).

In male rats there were increases in benign and combined benign/malignant adrenal pheochromocytomas which were statistically significant at the mid and low dose, but not at the highest dose (36 mg/kg/day). The incidences of benign pheochromocytomas exceeded the upper range (2-16%) of historical controls at all doses (there were no malignant pheochromocytomas reported). In male rats there were also increases in skin tumors (fibromas and papillomas) which were statistically significant at the low and mid doses, but not at the highest dose. Only 23 animals were microscopically examined at both these doses vs 50 in the controls and highest dose; however, if the denominators are adjusted to 50, assuming these type of lesions would be grossly detectable, the increases are no longer significant. The CPRC noted the lack of a significant response at the highest dose in male rats, for which there was no apparent explanation. The majority of the CPRC considered the tumor responses in both sexes to be marginal.

The dosing in the rats was considered to be adequate for both sexes; however, it was agreed that a higher dose could have been tolerated by the female rats.

Triclopyr was tested in several mutagenicity assays and found to be negative. There was however a positive dominant lethal assay in the rat; there was also a negative dominant lethal assay in the mouse.

Chlorpyrifos was considered as a structural analog; however it was noted that it inhibits acetyl cholinesterase (Triclopyr does not) and is therefore too toxic to be tested for carcinogenic potential at a high enough dose. Chlorpyrifos also is expected to be more readily metabolized than is Triclopyr. Chlorinated benzidines were also suggested as possible analogs.

Overall, the majority of the CPRC felt that the animal evidence was marginal (not entirely negative, but yet not convincing). Therefore Triclopyr was categorized as a Group D - not classifiable as to Human Carcinogenicity.

A. Individuals in Attendance at the meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

William Burnam	
Karl Baetcke	
Marcia Van Gemert	retired
Elizabeth Doyle	
Marion Copley	
Hugh Pettigrew	
Esther Rinde	
Richard Hill	

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Timothy McMahon[1]	
Yiannakis Ioannou	
Lori Brunsman[2]	
Lucas Brennecke[3] (PAI/ORNL)	

3. Other Attendees: Sanjivani Diwan (HED)

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[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Not present at meeting; signature indicates technical accuracy of statistics.

[3]Signature indicates concurrence with pathology report.

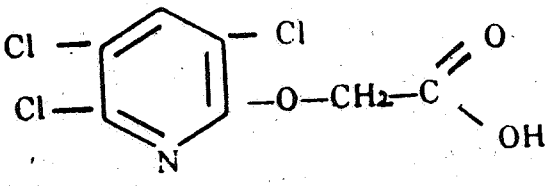
B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. McMahon, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. BACKGROUND

Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) is the active ingredient in GARLON herbicide formulations. The active ingredient Triclopyr is formulated as either the butoxyethyl ester or triethylamine salt of Triclopyr acid [GARLON 3a (3,5,6-trichloro-2-pyridinyloxyacetic acid, triethylamine salt) and GARLON 4a ((3,5,6-Trichloro-2-pyridinyl)oxy)acetic acid, n-butoxyethyl ester), respectively.] These salts of Triclopyr are used in formulations ranging from 8.8-44.4% for the triethylamine salt and from 16.5-61.6% for the butoxyethyl ester for the control of broadleaf and woody plants on utility rights-of-way, forests, industrial sites, and turf. Tolerances for the combined residues of Triclopyr and the metabolite 3,5,6-trichloro-2-pyridinol have been established under 40 CFR §180.417 for the raw agricultural commodities forage grasses and hay (500 ppm), milk (0.01 ppm), meat, fat and meat by-products (except liver and kidney) of cattle, goats, hogs, horses, and sheep (0.05 ppm), and liver and kidney of cattle, goats, hogs, horses, and sheep (0.5 ppm). Temporary tolerances have been approved for Triclopyr and its metabolite on rice grain and rice straw (0.5 and 8.0 ppm, respectively) and fresh water fish and shellfish (0.2 ppm). A temporary tolerance for Triclopyr only has been established for poultry, including poultry meat, fat and meat by-products (except kidney) at 0.2 ppm, in poultry kidney at 1.0 ppm, and in eggs at 0.05 ppm. An allowable residue level in potable water of 0.5 ppm was established in 1987, but is currently being re-evaluated by the Agency.

The issue of bioequivalency of the three chemical forms of Triclopyr (acid, triethylamine salt, and butoxyethyl ester) was addressed by the registrant through conduct of special studies with the triethylamine and butoxyethyl ester forms of Triclopyr. These studies, which included data on comparative disposition, plasma half-life, tissue distribution, hydrolytic cleavage under physiological and environmental conditions for Triclopyr triethylamine salt and Triclopyr butoxyethyl ester, were found to adequately address the issue of bioequivalency. In addition, subchronic toxicity studies conducted with each form supported the pharmacokinetic data in demonstrating bioequivalence.



STRUCTURE OF TRICLOPYR

CAS # 55335-06-3

PC Code# 116001

D. Evaluation of Carcinogenicity Data

1. Twenty-two Month Chronic Toxicity/Carcinogenicity Study in Mice.

Reference: Tsuda, S., Ebino, K., Ikeda, M., Harada, T., and Shirasu, Y. (1987): Triclopyr: 22-Month Oral Chronic Toxicity and Oncogenicity Study in Mice. Study conducted by The Institute of Environmental Toxicology, Tokyo, Japan, and submitted under MRID # 403566-01.

a. Experimental Design

In a chronic toxicity/carcinogenicity study, Triclopyr (98.0% a.i.) was administered in the diet to groups of male and female ICR mice at dose levels of 0, 50 ppm (**5.55 mg/kg/day** in males, **5.09 mg/kg/day** in females), 250 ppm (**28.6 mg/kg/day** in males, **26.5 mg/kg/day** in females) and 1250 ppm (**143 mg/kg/day** in males, **135 mg/kg/day** in females). Main test groups of 60 mice/sex/dose received diets for 95 weeks, while satellite groups of 40 mice/sex/dose were used for sacrifice of 10 mice/sex/dose at 26 and 52 weeks of treatment at the same dose levels. Tumorigenic evidence observed in this study is shown in the following table as extracted from the Qualitative Risk Assessment memorandum (Table 1):

b. Discussion of Tumor Data

There were no compound-related tumors observed in male mice.

Female mice had a significant increasing trend in mammary gland adenocarcinomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls (Table 1).

Table 1. Triclopyr - Jcl:ICR Mouse Study

Female Mammary Gland Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	50	250	1250
Adenocarcinomas	0/57	1/56	2/56	4[a]/53
(%)	(0)	(2)	(4)	(8)
p =	0.016*	0.496	0.243	0.051

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 32; also excludes both week 26 and week 52 interim sacrifice animals.

[a]First adenocarcinoma observed at week 32, dose 1250 ppm.

Note: There were no adenocarcinomas observed in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-Neoplastic Lesions and Other Findings:

At 143 mg/kg/day in males and 135 mg/kg/day in females, body weight gain in male mice was decreased 10.1% vs control for the 22-month study period, while body weight gain in female mice was decreased 10.6% for the 22-month study period. An increase in the incidence of thymic enlargement was observed in high dose male and female mice (9/59 vs 0/60), but there were no data on thymus weight. The report considered the enlarged thymus as unrelated to treatment based on the observation that those mice with enlarged thymuses were also found to have malignant lymphoma. However, the incidence of malignant lymphoma was not significantly different between control and treated male mice at any dose level. At 26 weeks of treatment, plasma BUN in male mice at 143 mg/kg/day was increased 25% vs control, while water consumption was increased an average of 25% at this dose beginning at week 13 of the study. In female mice, kidney weight was increased 10-16% at the 135 mg/kg/day dose, while urinary protein at the 135 mg/kg/day dose was also increased at week 52. However, there were no pathology data to support a true toxic effect on the kidney of males or females. Liver weight in male mice was increased by 17% at the 143 mg/kg/day dose level at week 26 only.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

At the high dose of 143 mg/kg/day in male mice and 135 mg/kg/day in female mice, body weight gain for the 22-month period was decreased by slightly greater than 10% in both sexes. Enlargement of the thymus appeared dose-related in male mice, reaching statistical significance at the mid and high dose levels. Increased incidence of mammary adenocarcinoma was observed in female mice at the 135 mg/kg/day dose level, and this increase appeared dose-related (see above statistical analysis). For the chronic toxicity portion of this study, the LEL is tentatively considered to be 143 mg/kg/day in male mice and 135 mg/kg/day in female mice, based on the decreased body weight gain. The NOEL is considered to be 28.6 mg/kg/day in male mice, and 26.5 mg/kg/day in female mice.

Additional support for the selection of the high dose in the chronic toxicity/carcinogenicity study is taken from a 28-day range-finding study in which male and female mice were exposed to Triclopyr in the diet at dose levels of 0, 200, 400, 800, 1600, and 3200 ppm (nominal doses of 30, 60, 120, 240, and 480 mg/kg/day). At the 3200 ppm dose, male mice were observed with single cell necrosis of the liver, significant increases in alkaline phosphatase, AST, and ALT, and enlargement of the liver with dark color. Centrilobular swelling and degeneration of hepatocytes was observed in a dose-dependent fashion at 800 ppm and above in male mice, along with mild increases in liver enzymes at 1600 ppm.

2. Two Year Chronic Toxicity/Carcinogenicity Study in Rats

Reference: Eisenbrandt, D.L., Firchau, H.M., Wolfe, E.L., and Landry, T.D. (1987): Triclopyr: 2-Year Dietary Chronic Toxicity-Oncogenicity Study in Fischer 344 Rats. Study conducted by Dow Chemical Company and submitted under MRID # 401077-01.

a. Experimental Design

In a chronic toxicity/carcinogenicity study, Triclopyr (98.0% a.i.) was administered in the diet to groups of male and female Fischer 344 rats (50/sex/dose) for 2 years at dose levels of 0, 3, 12, and 36 mg/kg/day. Additional groups of 10 rats/sex/dose received dietary Triclopyr at the same dose levels for 6 and 12 months. Tumorigenic evidence in this study is summarized below, as extracted from the Qualitative Risk Assessment memorandum (Tables 2, 3, and 4).

b. Discussion of Tumor Data

There were no significant increasing **trends** in tumor incidence for male rats. There were significant **pair-wise differences** vs control at 3 and 12 mg/kg Triclopyr in the incidence of adrenal gland benign pheochromocytomas and benign and/or malignant pheochromocytomas combined, and in the incidence of skin fibromas at 3 and 12 mg/kg, with $p < 0.05$ for all comparisons except the incidence of pheochromocytoma (benign + combined) at 12 mg/kg, where $p < 0.01$ vs control.

Female rats had significant increasing trends in mammary gland adenocarcinomas at $p < 0.05$ and in adenomas and/or adenocarcinomas combined at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 36 mg/kg/day dose group with the controls for mammary gland adenomas and/or adenocarcinomas combined at $p < 0.05$. There were no significant pair-wise comparisons or trends for the incidence of adrenal gland pheochromocytoma in female rats.

Table 2. Triclopyr - Fischer-344 Rat Study

Male Adrenal Gland Pheochromocytoma Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	3	12	36
Benign (%)	6/50 (12)	14[a]/49 (29)	17/50 (34)	12/50 (24)
p =	0.282	0.035*	0.008**	0.096
Malignant (%)	0/50 (0)	2[b]/49 (4)	3/50 (6)	0/50 (0)
p =	0.275	0.242	0.121	1.000
Combined (%)	6/50 (12)	16/49 (33)	20/50 (40)	12/50 (24)
p =	0.376	0.012*	0.001**	0.096

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

[a]First benign pheochromocytoma observed at week 85, 3 mg/kg/day.

[b]First malignant pheochromocytoma observed at week 95, 3 mg/kg/day.

Note: No animals in either of the interim sacrifice groups had any adrenal gland pheochromocytomas. Interim sacrifice animals are not included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Triclopyr - Fischer-344 Rat Study

Male Skin Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	3	12	36
Fibromas	1[a]/50	4/23[#]	5/23[#]	5/50
(%)	(2)	(17)	(22)	(10)
p =	0.297	0.032*	0.011*	0.102
Papillomas	0/50	0/23[#]	3/23[#]	3[b]/50
(%)	(0)	(0)	(13)	(6)
p =	0.108	1.000	0.029*	0.121

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

[a]First fibroma observed at week 93, dose 0 mg/kg/day.

[b]First papilloma observed at week 97, dose 36 mg/kg/day.

[#]Only those animals in the 3 and 12 mg/kg/day dose groups with macroscopic observations were examined microscopically for skin tumors.

Note: No animals in either of the interim sacrifice groups had any skin fibromas or papillomas. Interim sacrifice animals are not included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Triclopyr - Fischer-344 Rat Study

Female Mammary Gland Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	3	12	36
Adenomas	0/49	0/16[#]	0/19[#]	1[a]/50
(%)	(0)	(0)	(0)	(2)
p =	0.373	1.000	1.000	0.505
Adenocarcinomas	0/49	0/16[#]	0/19[#]	4[b]/50
(%)	(0)	(0)	(0)	(8)
p =	0.018*	1.000	1.000	0.061
Combined	0/49	0/16[#]	0/19[#]	5/50
(%)	(0)	(0)	(0)	(10)
p =	0.006**	1.000	1.000	0.030*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

[a]First adenoma observed at week 106, dose 36 mg/kg/day.

[b]First adenocarcinoma observed at week 92, dose 36 mg/kg/day.

[#]Only those animals in the 3 and 12 mg/kg/day dose groups with macroscopic observations were examined microscopically for mammary gland tumors.

Note: No animals in either of the interim sacrifice groups had any mammary gland tumors. Interim sacrifice animals are not included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-Neoplastic Lesions and Other Findings

Mortality in treated groups of male rats was lower than that in control. Cumulative mortality was stated as 50%, 32%, 26%, and 36% for control, low, mid, and high dose level male rats. Red cell count, hemoglobin, and hematocrit in male rats was numerically decreased at the high dose at 6, 12, and 24 months. Statistical significance was achieved for the decrease in red cells at 12 months, for hemoglobin at 6 months, and for hematocrit at 6 and 12 months. Absolute and relative kidney weight was significantly increased (10-17%) at the high dose in male rats, with an apparent dose-related trend at 12 months. Female rats showed an increased incidence of pigmentation of the proximal descending tubule at all dose levels compared to control, while male rats in the 6-month satellite group showed increased incidence of proximal tubule degeneration at the 12 and 36 mg/kg/day dose levels compared to control.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The apparent lack of biologically significant effects at the highest dose in this study brings into question the adequacy of dosing for carcinogenic potential. In support of the doses used in this study, the registrant cited data from a 90-day toxicity study in Fischer rats at dose levels of 0, 5, 20, 50, or 250 mg/kg/day Triclopyr in the diet (Accession # 073873). In that study, the effect level was considered as 20 mg/kg/day, based on an increased incidence of degeneration of the descending portion of the proximal tubule in male and female rats as well as a decrease in body weight gain in male rats. The severity but not the total incidence of proximal tubular degeneration increased with increasing dose in male rats, while female rats showed a less severe form of this pathology but an approximately equal incidence. Male rats showed a 6% increase in absolute kidney weight and an 8% increase in relative kidney weight at 50 mg/kg/day. At 250 mg/kg/day, body weight gain in male rats was reduced 17% and was reduced 7% in female rats. Liver ALT in male rats was increased 33%, with the observation of very slight eosinophilia in centrilobular hepatocytes. Absolute liver and heart weight were decreased 12-13% in male rats, and absolute testis weight was decreased 5% at the 250 mg/kg/day dose.

A 7% decrease in body weight gain, as was observed for male rats at 20 mg/kg/day in the 90-day study, is not convincing as an effect level, especially when food consumption was decreased between 9-16% at this same dose. The degeneration of the proximal tubules observed at the 50 mg/kg/day dose, being a dose-related phenomenon, might be more convincing as an effect of treatment, and is supported in part by the increase (6%) in kidney weight at this dose in male rats. This effect (degeneration of proximal tubules) was also observed in a separate 90-day toxicity study in rats

conducted with butoxyethyl ester Triclopyr (MRID # 422749-01) at 70 mg/kg/day, which, when corrected for acid equivalents, is approximately 50 mg/kg/day, the same dose used in the earlier study. The lesion itself was further described in that study as multiple foci of epithelial cell degeneration and regeneration, thickened tubular basement membranes, and interstitial fibrosis involving less than 10% of the descending portion of the proximal tubules.

In their meeting of 11/22/94, the Health Effects Division RfD/QA Peer Review Committee debated the possibility of setting the NOEL for the 2-year rat study at the 36 mg/kg/day dose level. However, since it appeared that the LEL in the 90-day study is at or near 50 mg/kg/day, the Committee considered the 36 mg/kg/day dose level in the 2-year study to be a threshold LEL in males and a NOEL in females. However, the 36 mg/kg/day dose level should be supported as an adequate dose for assessing the carcinogenic potential of Triclopyr, based on the statistical differences in mammary tumor incidence in female rats observed at this dose.

E. Additional Toxicology Data

1. Metabolism

Reference: Timchalk, C., Dryzga, M.D., and Kastl, P.E. (1988): Triclopyr: Tissue Distribution and Metabolism of ^{14}C-Labeled Triclopyr in Fischer 344 Rats. Study conducted by Dow Chemical Company, Midland, Michigan and submitted under MRID # 413530-01.

Data Summary: Disposition and metabolism of ^{14}C-Triclopyr was investigated in male and female rats at a low oral dose (3 mg/kg), repeated low oral doses (3 mg/kg x 14 days), and a high dose (60 mg/kg). Comparison of disposition data in intravenously dosed and orally dosed rats demonstrated that Triclopyr was well absorbed after oral administration. Excretion was relatively rapid at the low dose, with a majority of radioactivity eliminated in the urine by 24 hours. At 60 mg/kg, urinary elimination of ^{14}C-Triclopyr derived radioactivity was decreased in male and female rats from 0-12 hours, due to apparent saturation of renal elimination mechanisms. Fecal elimination of ^{14}C-Triclopyr derived radioactivity was a minor route of excretion, as was elimination via exhaled air. No significant effect was observed on metabolism or disposition of ^{14}C-Triclopyr from repeated low oral dosing in male or female rats. Residual ^{14}C-Triclopyr derived radioactivity was minimal in all dose groups, but measurable levels of tissue radioactivity were detected in perirenal fat of both sexes and ovaries of female rats which apparently increased with dose. Thus, potential accumulation of ^{14}C-Triclopyr derived radioactivity may occur in these tissues.

Urinary metabolites of ^{14}C-Triclopyr were isolated and identified by HPLC and GC/MS. Unmetabolized parent chemical represented >90% of urinary radioactivity, with the remainder accounted for by the metabolite 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), and possible glucuronide and/or sulfate conjugates of 3,5,6-TCP.

Plasma elimination following intravenous administration of ^{14}C-Triclopyr was consistent with a one-compartment model with an elimination half-life of 3.6 hrs. and zero-order kinetics from 0-12 hours at the 60 mg/kg dose. Kinetic parameters were optimized using SIMUSOLV modeling software. The model showed an apparent "flip-flop" phenomenon, in which absorption at the 3 mg/kg dose was rate limiting in elimination of ^{14}C-Triclopyr derived radioactivity, but renal excretion was saturated and therefore limiting in elimination of ^{14}C-Triclopyr derived radioactivity at the 60 mg/kg dose.

2. Mutagenicity

Mutagenicity data are available for both the acid form and butoxyethyl ester form of Triclopyr. These studies are summarized below.

a. Butoxyethyl Ester Triclopyr

i) Reference: Samson, Y.E. and Gollapudi, B. (1990): Evaluation of Triclopyr Butoxyethyl Ester (Triclopyr BEE) in the Ames Salmonella / Mammalian-Microsome Bacterial Mutagenicity Assay. Study conducted by DowElanco and submitted under MRID # 417322-02.

Under the conditions of this study, Triclopyr BEE was found to be non-mutagenic in four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) in the presence or absence of metabolic activation at the concentrations tested (50-5000 µg/plate).

Core Classification: acceptable (HED document # 009299)

ii) Reference: Samson, Y.E. and Gollapudi, B. (1990): Evaluation of Triclopyr Butoxyethyl Ester (Triclopyr BEE) in the Mouse Bone Marrow Micronucleus Test. Study conducted by DowElanco and submitted under MRID # 417471-01.

Triclopyr BEE was not clastogenic in the mouse micronucleus test at the dose levels tested (60, 200, and 600 mg/kg).

Core Classification: acceptable (HED document # 009299)

iii) Reference: Gollapudi, B. (1990): Evaluation of Triclopyr Butoxyethyl Ester (Triclopyr BEE) in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay (mrid # 41747102).

Triclopyr BEE did not cause DNA damage or inducible repair in the rat hepatocyte unscheduled DNA synthesis assay at the concentrations of test article used in this study (1.0-1000 µg/ml).

Core Classification: acceptable (HED document # 009299)

b. Triclopyr Free Acid

i) Mutagenicity Test on Triclopyr (Dowco 233) in Bacterial Systems. Study performed by the Institute of Environmental Toxicology and submitted under MRID # 00038408.

The mutagenicity of Triclopyr was evaluated in a recombination repair system using rec- assay mutant (H17) and recombination repair deficient mutant (M45) of B. subtilis and was also tested in the reverse mutation assay using *Salmonella* strains TA 98 and TA 100. Concentrations used in the rec- assay were 20-2000 μg/disk, and 1-5000 μg/plate in the reversion assay.
In the rec- assay, there was no evidence of growth inhibition for the repair competent or repair deficient bacterial strains employed. In the reversion assay, there were no increases in number of revertant colonies in the absence or presence of liver S-9 for the strains of *Salmonella* employed.

Classification: acceptable[4] (HED document # 001456; 001838)

ii) Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Dowco 233. Study performed by Huntington Research Centre and submitted under MTID # 00031939.

In this study, the mutagenic potential of Triclopyr (98.0% a.i.) was assessed in *Salmonella* tester strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 in the absence and presence of metabolic activation (rat liver S-9). Concentrations used were 10, 1000, and 10,000 μg/plate. There were no significant increases in the number of revertant colonies for any of the tester strains employed in this study in the absence or presence of metabolic activation.

Classification: acceptable (HED document # 001457; 001838)

iii) Dominant Lethal Evaluation of Dowco 233 in CF-1 Mice. Study performed by Dow Chemical Co., Midland, Michigan and submitted under MRID #'s 00028996, 00028997, 00052986, 00071796.

In this study, groups of 30 male mice were maintained on dietary levels of Triclopyr of 0, 3, 15, or 70 mg/kg/day for 9 consecutive weeks. Immediately following treatment, each male was mated to 4 untreated mature virgin females for 7 consecutive days. Two of the 4 females in each group were held for the dominant lethal study. Ten days following the last day of cohabitation, females were sacrificed and uteri examined for live and dead implants. There were no significant toxic effects observed in treated male mice,

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[4]This assay by itself is unacceptable, due to less than required number of Salmonella strains used, but together with the Ames Test described in section b.ii, and the results of the Salmonella Assay with the Butoxyethyl Ester of Triclopyr, it becomes acceptable.

and no significant differences in body weights. There were no significant effects on fertility index, average number of implantations, average number of resorptions, average resorption rate, or average litter size in any of the untreated female mice bred to treated males at all dose levels of Triclopyr tested.

Classification: Unacceptable, since dosing was not high enough (HED Document #'s 001439, 001455, 001838).

iv) Evaluation of Triclopyr in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay. Study performed by Dow Chemical Co., Midland, Michigan and submitted under MRID # 400557-02.

In this study, rat primary hepatocyte cultures were exposed to Triclopyr at concentrations of 5 x 10^{-3}, 1.56 x 10^{-3}, 5 x 10^{-4}, 5 x 10^{-5}, 1.56 x 10^{-5}, and 5 x 10^{-6} M for 18 hours in the presence of 10 μCi/ml ^{3}H-thymidine. Triclopyr failed to induce any increase in net nuclear grain counts at any of the concentrations tested. Hepatocyte toxicity was demonstrated at 5 x 10^{-3} Triclopyr.

Classification: acceptable (HED Document # 005916).

v) Acute and subacute in vivo host-mediated assay for mutagenesis (1973). Study conducted by Litton Bionetics Inc., and submitted under MRID # 00057085.

In a host-mediated assay, Triclopyr was administered orally at doses of 0.7, 7.0, and 70.0 mg/kg to groups of 10 male ICR random bred mice. In the acute test, the indicator organism (*Salmonella* TA-1530, *Salmonella* G-46, and *Saccharomyces* D-3) was injected i.p. immediately after administration of test material. In subacute tests, the indicator organism was injected 1/2 hour after the last of 5 administrations of test material (5 times at 24 hour intervals). Intraperitoneal fluid was recovered, diluted, and plated for determination of revertants and recombinants. Triclopyr in this study induced no significant increases over negative control in mutant or recombinant frequencies at the dose levels used in this study.

Classification: acceptable (HED Document # 001440)

vi) Acute and subacute in vivo cytogenetic study in rats (1973). Study conducted by Litton Bionetics and submitted under MRID # 00057086.

In this study, Triclopyr was administered to groups of 5 Sprague-Dawley rats as single doses of 0.7, 7.0 , and 70.0 mg/kg, or for 5 days to additional groups of 5 rats at the same dose

levels. In the single dose study, rats were sacrificed at 6, 24, and 48 hours after test administration, while in the repeated dose study, rats were sacrificed at 5 days after the last dose. Examination of bone marrow cells for chromosomal aberrations from the acute and subacute groups showed no cells with chromosomal aberrations.

Classification: acceptable (HED Document # 001440)

vii) Dominant Lethal Assay in rats for Mutagenesis. Study conducted by Litton Bionetics and submitted under MRID # 00057087.

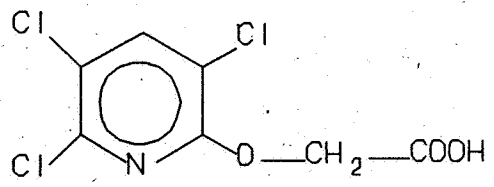
In this study, Triclopyr at doses of 0.7, 7.0, and 70.0 mg/kg, triethylene melamine (positive control) at a dose of 0.3 mg/kg, or negative control (corn oil plus saline) were administered orally to separate groups of 10 male Sprague-Dawley rats. Males were sequentially mated to 2 untreated females per week for 7 weeks. Females were killed at 14±2 days after mating. There was an apparent decrease in mating index during week 1 at the 7 and 70 mg/kg dose levels. A trend towards an increase in average number of resorptions was evident at the 7 and 70 mg/kg dose levels, but statistical significance (by t-test) was apparent only at week 4 at the 7 mg/kg dose, week 5 at the 70 mg/kg dose, and week 7 at the 70 mg/kg dose. Statistical comparison by t-test is not appropriate in this type of experimental design. The proportion of females with one or more dead implantations also appeared increased at the 70 mg/kg dose level over negative control. The ratio of dead implants to total implants was also increased at the 7 and 70 mg/kg dose levels, but the increases were numeric in most of the cases.

Classification: acceptable (HED Document # 001440)

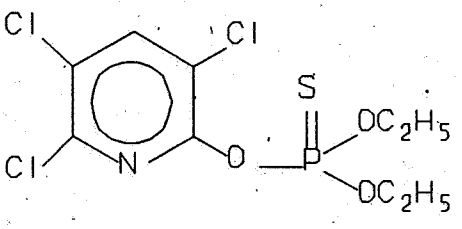
Based on the positive effects in the rat dominant lethal assay above (MRID # 00057087), OPP required a heritable translocation study to be performed. The Registrant began the study with mice and submitted litter size data only (MRID # 00029007). The heritable translocation study (or portion of a larger test including the unacceptable mouse dominant lethal test) was discontinued, since the Registrant concluded that the mouse dominant lethal portion (MRID # 00071796) did not have a dominant lethal effect. Based only on the litter information, there appeared to be a suggestive effect at 15 mg/kg/day, but there was no information to confirm partial or complete sterility. This suggestive result and the rat dominant lethal results need follow-up. A properly conducted and reported heritable translocation study in rats is an appropriate follow-up based on this evidence.

3. Structure-Activity Considerations

Triclopyr bears a structural resemblance to the insecticide Chlorpyrifos. The two structures are shown below, and illustrate that the difference in structure is in the attached side chain of the pyridine ring. It is noted that the 3,5,6-trichloro-2-pyridinol metabolite is formed from both chemicals. Chlorpyrifos was considered as a structural analog; however it was noted that it inhibits acetyl cholinesterase (Triclopyr does not) and is therefore too toxic to be tested for carcinogenic potential at a high enough dose[5]. There are also differences between the two chemicals from a metabolic point of view; in Chlorpyrifos, the pyridinol moiety is linked by an ester linkage, and is expected to be readily hydrolyzed. Triclopyr, on the other hand, has an ether linkage which is expected to be much more difficult to metabolize. Chlorinated benzidines were also suggested as possible analogs.



Triclopyr



Chlorpyrifos

[5]The Health Effects Division RfD/Quality Assurance Committee, in their meeting of September 9, 1993, concluded that the doses used in the chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice were adequate for assessment of carcinogenic potential of chlorpyrifos, and that the chemical did not alter the spontaneous tumor profile in the strains of rats and mice tested. Chlorpyrifos was therefore classified as a "Group E" carcinogen (no evidence of carcinogenicity).

F. Weight-of-Evidence Consideration

The Health Effects Division Carcinogenicity Peer Review Committee is asked to consider the following toxicology data in determining the carcinogenic potential of Triclopyr:

1) In the mouse carcinogenicity study, administration of Triclopyr in the diet for 95 weeks was associated with a significant increasing trend in mammary gland adenocarcinomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

2) In the rat chronic toxicity/carcinogenicity study, administration of Triclopyr in the diet for 105 weeks was associated with significant **pair-wise differences** in male rats vs control at 3 and 12 mg/kg Triclopyr in the incidence of adrenal gland benign pheochromocytomas and benign and/or malignant pheochromocytomas combined, and in the incidence of skin fibromas at 3 and 12 mg/kg, with $p < 0.05$ for all comparisons except the incidence of pheochromocytoma (benign + combined) at 12 mg/kg, where $p < 0.01$ vs control.

Female rats had significant increasing trends in mammary gland adenocarcinomas at $p < 0.05$ and in adenomas and/or adenocarcinomas combined at $p < 0.01$. There was a significant pair-wise difference at 36 mg/kg/day vs control in the incidence of mammary gland adenomas and/or adenocarcinomas combined at $p < 0.05$.

3) Mutagenicity testing conducted with both the acid form and butoxyethyl ester form of Triclopyr showed a lack of mutagenic effects with the exception of one study (dominant lethal assay), in which a trend towards an increase in average number of resorptions, proportion of females with one or more dead implantations, and ratio of dead implantations to total implantations was observed at doses of 7 and 70 mg/kg. Increases were numeric in most cases, and statistical significance, where noted, may have been based on an inappropriate statistical method for the experimental design of the study.

4) Triclopyr is structurally related to Chlorpyrifos. Experimental data on the carcinogenicity of Chlorpyrifos has demonstrated no evidence of carcinogenicity for Chlorpyrifos; however, it was noted that Chlorpyrifos inhibits acetyl cholinesterase (Triclopyr does not) and is therefore too toxic to be tested for carcinogenic potential at a high enough dose.

5) Triclopyr has not been previously classified for carcinogenicity by the Health Effects Division Carcinogenicity Peer Review Committee.

6) In the two year rat study, only 16 low dose female rats and 19 mid dose female rats were examined microscopically for the presence

of mammary tumors. The registrant was asked if slides for the remaining rats were available. The registrant replied that while the tissues were available, microscopic examination of mammary tissue was conducted in the low and mid dose groups only if the animal died or was sacrificed prior to study termination, or if a gross lesion was observed. The registrant further stated that the likelihood of seeing a proliferative lesion in the standard sample in the absence of a gross lesion was low[6].

The registrant's response was evaluated by Dr. Lucas H. Brennecke, Pathology Consultant for the Health Effects Division. Dr. Brennecke was in agreement with all of the points mentioned in the registrant's response, but added that "when a tissue is identified as a "target" tissue or "potential target" tissue based on a comparison of control and high dose animals, then that tissue in the low and intermediate dose groups is normally prepared and evaluated. It is possible that proliferative changes may be seen microscopically which were not visible grossly[7]."

The CPRC did not consider that submission of the additional slides will alter the original interpretation of the carcinogenic potential of Triclopyr.

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[6]Merlyn Jones to Tim McMahon, Sept. 08, 1995.

[7]Lucas Brennecke to Esther Rinde, Oct. 08, 1995.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The decision to classify Triclopyr as a Group D was based on increases in mammary tumors in both the rat and mouse, and adrenal pheochromocytomas in the male rat, which the majority of the CPRC believed to be only marginal. There was no apparent concern for mutagenicity although a data gap for a heritable translocation assay was identified. Data from structural analogs did not provide additional support.

A classification of Triclopyr as a Group C was also considered, since the same tumor type (mammary) appeared in both species; there were also adrenal pheochromocytomas in male rats, all significant tumor incidences exceeded historical controls, both studies were performed at adequate doses for carcinogenicity testing and neither had any flaws. However, the consensus of the CPRC was to classify Triclopyr as a Group D, based on what was considered only a marginal response and the absence of additional support from structural analogs or genotoxicity.

The CPRC did not consider any further basic mutagenicity testing to be warranted; however, a heritable translocation assay is indicated as an appropriate follow-up for additional testing. Discussion between the Registrant and OPP is needed before initiation of the follow-up study.

H. Induces Cancer Call -- Triclopyr

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to Triclopyr resulted in an increased incidence of mammary tumors (malignant) in the female ICR mouse and mammary combined adenoma/carcinoma (mainly benign) in the female F344 rat. There were also increases in benign adrenal pheochromocytomas (mainly benign) in the male rat. All of these increases were considered to be marginal.

There does not appear to be evidence of genotoxicity for Triclopyr; however, based on suggestive positive effects in a rat dominant lethal assay, a properly conducted and reported heritable translocation study is indicated as an appropriate follow-up for additional testing.

The Committee agrees that Triclopyr induces cancer in animals.